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Docket No. 377882000800

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Diane Blevins

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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In the application of:

Gary VAN NEST, et al.

Serial No.: 09/642,492

Filing Date: August 18, 2000

For: METHODS OF MODULATING AN IMMUNE RESPONSE USING IMMUNOSTIMULATORY SEQUENCES AND COMPOSITIONS FOR USE THEREIN

Examiner: S. Foley

Group Art Unit: 1648

**DECLARATION OF GARY VAN NEST, PH.D.
PURSUANT TO 37 C.F.R. § 1.132**

Box AF
Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Gary Van Nest, Ph.D., declare as follows:

1. I currently reside at 639 Skyline Drive, Martinez, California 94553.

2. I am an inventor named in the above-referenced patent application, and am familiar with the written communication from the Patent Office dated April 23, 2002.

3. Described herein are additional results from experiments, performed by me or under my direction, which are from controls performed along with the experiments described in Example 1 in the patent specification. These results support the difference in the immune

response to an antigen (*i.e.*, second antigen) following the claimed method (administration of (i) an ISS-containing polynucleotide in proximate association with a first antigen and (ii) a second antigen) from the immune response to the antigen (*i.e.*, second antigen) following the control method (administration of (i) an ISS-containing polynucleotide and (ii) the antigen (*i.e.*, second antigen)).

4. As described in Example 1 on page 50 of the specification, sets of mice were immunized with either 1 μg βgal, 1 μg βgal mixed with 1 μg AIC or 1 μg βgal mixed with 10 μg AIC. AIC denotes a conjugate of the antigen Amb a 1 and ISS-containing polynucleotide of SEQ ID NO:1. In addition to what is described in Example 1 of the specification, control sets of mice were immunized intradermally three times at two-week intervals with either 1 μg βgal mixed with 1 μg ISS or 1 μg βgal mixed with 10 μg ISS (admixture controls). Two weeks after the second and third immunizations, βgal-specific IgG1 and IgG2a responses were determined by ELISA as described in Example 1. Four weeks after the third immunization, mice were sacrificed, and spleen cell IFNγ and IL-5 responses to βgal were determined by ELISA as described in Example 1.

5. The results of this experiment with 1 μg βgal, 1 μg βgal mixed with 1 μg AIC, and 1 μg βgal mixed with 10 μg AIC are presented in Figure 1 and Tables 2 and 3 of the specification. The results of the admixture controls of this experiment with 1 μg βgal mixed with 1 μg ISS and with 1 μg βgal mixed with 10 μg ISS are herein presented in Exhibits A and B. The experimental data presented in the specification was calculated as arithmetic means as compared to the experimental data presented in Exhibits A and B which was calculated as geometric means.

6. After both the second and third immunizations, the antibody response to immunization with βgal alone was predominantly an IgG1 response, consistent with a Th2 response. Administration of βgal in an admixture with 1 or 10 μg of ISS-containing polynucleotide (admixture controls) resulted in a shift in the immune response away from a Th2 response and toward a Th1 response (Exhibit A). Co-administration of one or ten μg AIC (the ISS-containing polynucleotide linked to the antigen Amb a 1) with βgal modulated the βgal-specific immune response compared to the administration of βgal mixed with an ISS-containing polynucleotide (Exhibit A) and modulated the βgal-specific immune response compared to the

administration of βgal alone (specification, Figure 1 and Table 2). For example, modulation of a Th1 immune response was demonstrated by the increased IgG2a response to βgal after the second immunization with AIC as compared to the IgG2a response seen with βgal alone and as compared to the IgG2a response seen with βgal mixed with an ISS (admixture control) as depicted in Exhibit A.

7. As depicted in Table 3 of the specification and in Exhibit B, spleen cells from mice immunized with βgal alone secreted a relatively low level of IFNγ and a relatively high level of IL-5 in response to βgal. These cytokine responses are indicative of a Th2 response, consistent with the antibody responses discussed above. Administration of βgal in an admixture with 1 or 10 μg of ISS-containing polynucleotide resulted in a shift in the immune response away from a Th2 response and toward a Th1 response as indicated by an increased IFNγ and a decreased IL-5 in response to βgal (Exhibit B). Co-administration of βgal with 1 or 10 μg of AIC increased the IFNγ response and decreased the IL-5 response in response to βgal by the spleen cells (specification Table 3), again demonstrating a modulation of an immune response to βgal when the βgal is administered with AIC as compared to when the βgal is administered with an ISS-containing polynucleotide.

8. The data from this experiment, presented herein and in Example 1, demonstrate the difference in the immune response to an antigen (βgal) when administered with an ISS-containing polynucleotide (ISS) and when administered with an ISS-containing polynucleotide in proximate association with a different antigen (AIC). These admixture control results support the discovery that administration of a second antigen with an ISS-containing polynucleotide in proximate association to a first antigen results in a modulation of an immune response to the second antigen.

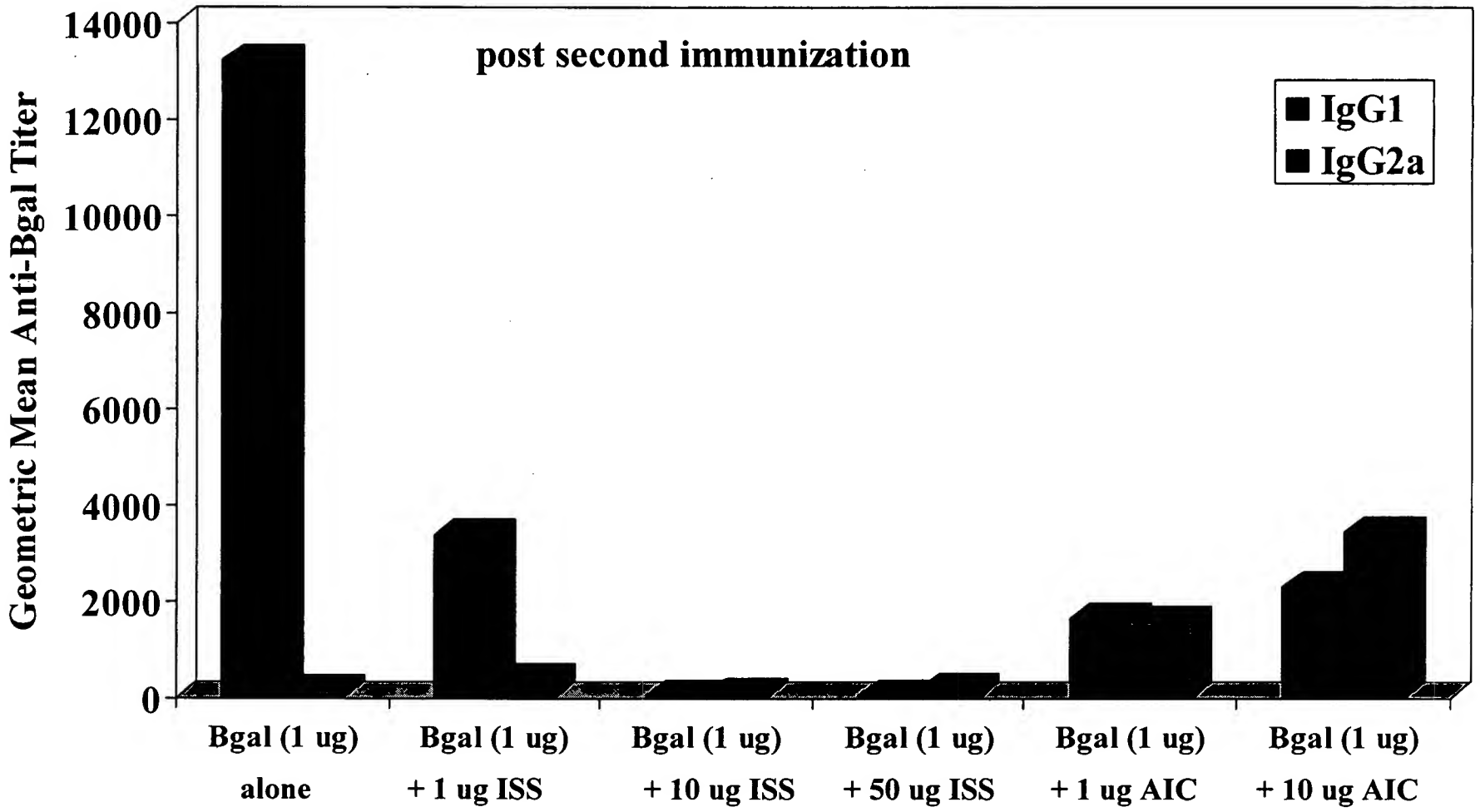
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

October 22, 2002
Date

Gary Van Nest

Anti-βgal Antibody Responses of Mice Co-administration of βgal with ISS or AIC

EXHIBIT A



Anti-βgal Cytokine Responses of Mice Co-administration of βgal with ISS or AIC

EXHIBIT B